INFECTION AND IMMUNITY, Apr. 1983, p. 303-309
0019-9567/83/040303-07$02.00/0

Vol. 40, No. 1

Corncob Formation Between *Fusobacterium nucleatum* and *Streptococcus sanguis*

P. LANCY, JR.,[1] J. M. DIRIENZO,[1]* B. APPELBAUM,[1] B. ROSAN,[1] AND S. C. HOLT[2]

Department of Microbiology, School of Dental Medicine and Center for Oral Health Research, University of Pennsylvania, Philadelphia, Pennsylvania 19104,[1] and Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003[2]

Received 12 November 1982/Accepted 4 January 1983

Corncob formation in dental plaque was believed to be limited to strains of *Bacterionema matruchotii* and *Streptococcus sanguis*. We observed recently that strains of *Fusobacterium nucleatum* also interacted with *S. sanguis* to form corncobs. Since the fusobacteria are among the first anaerobic filaments to colonize subgingival plaque, these interactions could serve as a connecting link between the transformation of supra- to subgingival plaque. To further characterize these interactions, quantitative in vitro studies of the kinetics of corncob formation of the fusobacteria were undertaken. These studies indicated that fewer streptococci were needed to saturate *F. nucleatum* strain 364 compared to strain 10953. Corncob formation with both strains was enhanced with increasing pH up to pH 8, at which point autoaggregation of the streptococci occurred. Variation in ionic strength and divalent cations had little effect on the interaction, and EDTA suppressed aggregate formation only slightly. Detergents at concentrations above 0.05% also inhibited corncob formation. Electron micrographs suggested that attachment of the cocci to the fusiforms was mediated through localized tufts of fimbriae, as they are in the *Bacterionema* system. However, although both trypsin and heat treatment of the streptococci inhibited corncob formation with fusobacteria, the effects were not as complete as those seen in *Bacterionema* species. Unlike the *Bacterionema* model, trypsin and heat treatment of the fusobacteria resulted in inhibition of corncob formation. These results suggest that several different receptors may be involved in corncob formation.

Dental plaque is an accumulation of oral bacteria in an adherent matrix of polysaccharide and other bacterial and host products on tooth surfaces. Although the quantitative microbial composition is highly variable, plaque formation is usually characterized by an ordered succession of cocci followed by filamentous forms (1, 11, 21). A dominant organism in the initial colonization of the tooth surface is *Streptococcus sanguis* (3–5); this appears to be associated with the affinity of the organism for the salivary glycoproteins in the acquired pellicle on the tooth surface (27). The streptococci in these nidi multiply to form microcolonies (23) which subsequently are "invaded" by a mixture of gram-positive rods, anaerobic gram-negative cocci, and filaments characteristic of mature supragingival plaque (11). A number of environmental factors, including alterations in the redox potential, appear to play a part in the appearance of filaments (21). An especially important factor may be the specific interaction of the surface of the filaments with the surfaces of the streptococci. The most common species of oral streptococci involved in these reactions also appears to be *S. sanguis* (23).

S. sanguis forms aggregates with *Actinomyces* spp. (2, 6, 7, 12), *Streptococcus mutans* (24), *Bacteroides* spp. (25), and *Bacterionema* sp. (8–10, 14–17, 26). The aggregates with *Bacterionema* sp. are of particular interest because they form highly specific morphological units referred to as "corncobs" (8). These units consist of a filamentous organism surrounded by adherent cocci (8, 10, 14–17) and resemble an ear of corn. Although the corncob was originally thought to be associated only with specific strains of *S. sanguis* and *Bacterionema matruchotii*, we have found that corncobs are also formed between *S. sanguis* and *Fusobacterium nucleatum*. The fusobacteria are major inhabitants of subgingival plaque (13), and corncob formation with fusobacteria may serve as a connecting link between supra- and subgingival plaque. Study of the factors affecting corncob formation with the fusobacteria could be important for understanding plaque maturation and for developing strategies of prevention.

MATERIALS AND METHODS

Strains, media, storage, and growth conditions. The streptococcal strains used in this study, CC5A and G9B, have been described previously (1, 9). Streptococci were grown in brain heart infusion broth at 37°C. *F. nucleatum* 10953 and *B. matruchotii* 14266 were obtained from the American Type Culture Collection, Rockville, Md., and *F. nucleatum* 364 was obtained from S. S. Socransky, Forsyth Dental Center, Boston, Mass. The *Fusobacterium* strains were grown in 100 ml of brain heart infusion broth supplemented with 0.2% yeast extract, 0.05% L-cysteine, and 0.5% sodium bicarbonate under anaerobic conditions (Gas-Pak; BBL Microbiology Systems, Cockeysville, Md.).

Culture harvesting. The streptococcal cultures were harvested by centrifugation at 9,000 × *g* for 15 min at 4°C. The cell pellets were suspended in 1/10 the original volume in 0.15 M NaCl and pelleted. This procedure was repeated once more, and the final pellet was suspended in 0.15 M NaCl to a value of 1,000 Klett units at 470 nm. The *Fusobacterium* cultures were treated similarly, except that centrifugation was at 12,000 × *g*. The cell suspensions were stored on ice until used (maximum, 7 days). No changes in the ability of the cells to form corncobs were observed during this time.

Radioactive labeling conditions. Streptococci were labeled with [*methyl*-^{3}H]thymidine at a concentration of 1 to 2 μCi/ml of brain heart infusion broth as described previously (1, 9). The specific activity of the labeled streptococci was approximately 2.4×10^4 cells/cpm (CC5A) and 1.3×10^5 cells/cpm (G9B).

Corncob assay procedure. The reaction mixture consisted of 50 μl of *Fusobacterium* suspension (containing approximately 5×10^7 fusobacteria), 175 μl of streptococcal suspension (containing 1.8×10^9 streptococci), and 1,775 μl of buffer in a polystyrene 12- by 75-ml test tube (W. Sarstedt, Inc., Princeton, N.J.). The buffer was either 0.05 M Tris-hydrochloride (pH 8.0) or 0.05 M sodium phosphate (pH 7.0); the reaction mixtures were incubated for 1 h at 37°C on a rocking platform mixer (LabQuake; Labindustries, Inc., Berkeley, Calif.). The presence of corncobs was assayed both microscopically and quantitatively by retention on 5-μm filters (Nucleopore Corp., Pleasanton, Calif.) as described previously (9). Kinetic data were plotted as the mean values calculated from triplicate samples.

Effects of pH, salts, and detergents on corncob formation. Sodium, potassium, magnesium, and calcium, as either chloride or acetate salts, were tested for their effects on corncob formation when added to the reaction mixture in the range of 100 to 500 mM. EDTA was also tested in the same range. Ionic effects were evaluated in both sodium phosphate and Tris-hydrochloride buffers with buffer concentrations ranging from 10 to 500 mM. The effects of pH and detergents (sodium dodecyl sulfate and Triton X-100) in the concentration range of 0.01 to 1% (vol/vol) on corncob formation were determined.

Electron microscopy. For transmission electron microscopy, cells and corncobs were fixed by a two-step regimen. Entire corncob reaction mixtures were made 0.25% in redistilled glutaraldehyde (Baker, Thomas Scientific Co., Philadelphia, Pa.) and incubated at 4°C overnight in the dark. The mixtures were then made 2.5% in glutaraldehyde. The fixed corncobs were enrobed in warm Nobel agar, and the resulting pellets were washed four times in cold phosphate buffer and postfixed in 2% (wt/vol) osmium tetroxide for 1 h at 4°C. The pellets were then washed once in phosphate buffer, dehydrated through a graded ethanol series, and embedded in Epon. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate (20), and examined in a JOEL 100S transmission electron microscope.

Samples for scanning electron microscopy were prepared as described previously (19) and examined in a JOEL 25S scanning electron microscope.

Saliva-coated hydroxyapatite adherence assays. Saliva-coated hydroxyapatite adherence assays were performed as previously described (1). Briefly, [^{3}H]thymidine-labeled *S. sanguis* G9B cells were used as reference cells in competition assays. Increasing amounts of unlabeled *F. nucleatum* 364 or 10953 cells were added to a fixed concentration of G9B cells in the presence of saliva-coated hydroxyapatite beads, and the amount of radiolabeled cells adhering to the beads was quantitated. The results were expressed as a function of the percentage of bound labeled cells in the total cell mixture, relative to control assays with no fusobacteria added.

Heat and trypsin treatment. Samples of cells to be heat treated were centrifuged in a Microfuge (Fisher Scientific Co., Pittsburgh, Pa.) for 1 min at 13,000 × *g*. The pellets were resuspended in an equal volume of buffer and placed in a 70°C water bath. After appropriate time periods, tubes were removed and centrifuged once more, the pellets were resuspended in fresh buffer, and the suspensions were assayed for ability to form corncobs. Samples of cells to be trypsin treated were similarly pelleted. The pellets were resuspended in an equal volume of 0.05 M Tris-hydrochloride buffer (pH 8.0) containing 0.5 mg of trypsin per ml (Sigma Chemical Co., St. Louis, Mo.) and incubated at 37°C in a water bath. At the end of the incubation period, the tubes were centrifuged, and the pellets were washed three times with buffer and resuspended in buffer containing 0.5 mg of soybean trypsin inhibitor (Sigma) per ml. Controls indicated that the inhibitor had no effect on corncob formation. The tubes were incubated for 30 min at 37°C and centrifuged, and the pellets were washed as described above and resuspended to the original volume before corncob assay.

RESULTS

Morphological observations. When strains of *F. nucleatum* were mixed with *S. sanguis* and examined by phase-contrast microscopy, corncob formation similar to that observed with *Bacterionema* sp. and *S. sanguis* was seen (Fig. 1) (9). The streptococci were arranged along the surface of the filament, and at saturation, the filament was completely obscured. At subsaturating conditions, the arrangement of the streptococci was random. Scanning electron micrographs revealed that the association of *F. nucleatum* and *S. sanguis* (Fig. 2A) had a morphology similar to that seen in the *Bacterionema* model. Thin sections of corncobs shown in Fig.

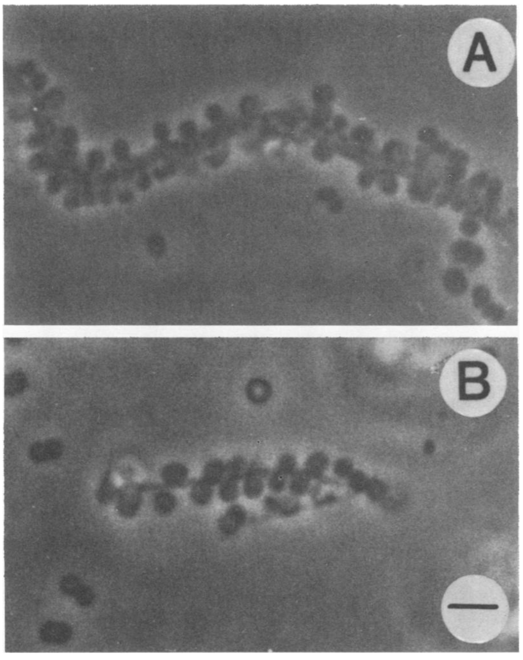

FIG. 1. Phase-contrast micrographs of in vitro corncobs formed between (A) *S. sanguis* CC5A and *B. matruchotii* 14266 and (B) *S. sanguis* CC5A and *F. nucleatum*. Bar, 2 μm.

2B suggest that the streptococci bind to the filament through the localized "fuzz" (fimbriae) which is found on the surface of *S. sanguis* CC5A (16). The fimbriae appear to be in direct contact with the outer surface of *F. nucleatum*.

Kinetics of coaggregation. The time course of the reaction of the fusobacteria with *S. sanguis* CC5A is shown in Fig. 3. The reaction was extremely rapid; within 5 to 10 min, 50% of the total streptococci which bound at saturation were aggregated. An incubation time of 1 h was chosen for the standard assay.

The addition of increasing numbers of streptococci to a constant number of fusobacteria (8.5 $\times 10^7$ cells) resulted in a typical saturation curve (Fig. 4). Saturation of the fusobacteria was obtained at an input of 5×10^9 streptococci. A comparison of the saturation kinetics of the two strains of *F. nucleatum* revealed that strain 364 appeared to bind fewer streptococci than strain 10953. These kinetic experiments were performed in phosphate buffer at pH 7.0. When the experiments were repeated in Tris-hydrochloride buffer at pH 8.0, the strain differences were abolished (data not shown). A more detailed examination of this phenomenon demonstrated that the kinetic differences were due to the pH of the reaction; at a pH of 7.5 or greater, in either phosphate or Tris buffer, the saturation curves for both strains of fusobacteria were identical. Corncob formation appeared to increase with increasing pH, up to pH 8, in either sodium phosphate or Tris-hydrochloride buffer. At higher pH values, the streptococci showed extensive aggregation, whereas acidic conditions suppressed coaggregation.

Effects of inorganic compounds and detergents on coaggregation. The addition of sodium, potassium, magnesium, or calcium, as either the chloride or acetate salts, had no effect or resulted in erratic effects on coaggregation. The various salts were tested at concentrations ranging from 100 to 500 mM, and, generally, increasing salt concentrations increased aggregation of the streptococci in the controls. The magnesium salts appeared to show a slight stimulation of coaggregation, and chelating agents such as EDTA demonstrated only a slight suppression of coaggregation. Ionic effects were also minimal, since changes in the concentration of either

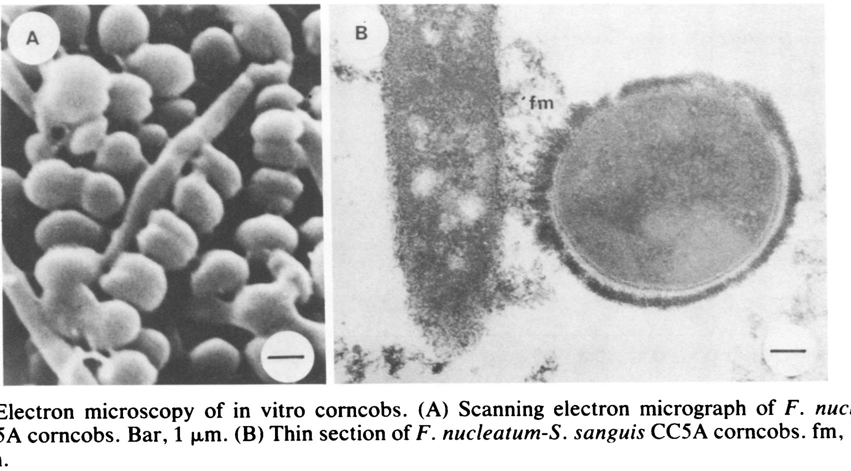

FIG. 2. Electron microscopy of in vitro corncobs. (A) Scanning electron micrograph of *F. nucleatum-S. sanguis* CC5A corncobs. Bar, 1 μm. (B) Thin section of *F. nucleatum-S. sanguis* CC5A corncobs. fm, Fimbriae. Bar, 0.1 μm.

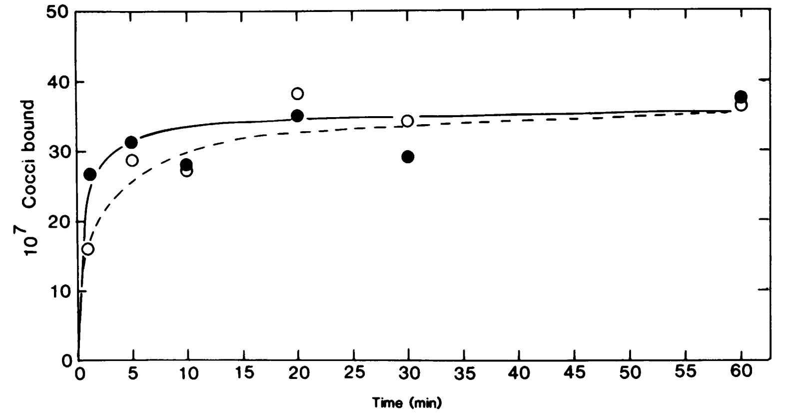

FIG. 3. Number of *S. sanguis* CC5A cells bound to *F. nucleatum* as a function of time of incubation. Open circles, *F. nucleatum* 364; closed circles, *F. nucleatum* 10953.

sodium phosphate or Tris-hydrochloride ranging from 10 to 500 mM neither stimulated nor depressed coaggregation. At concentrations above 0.05%, detergents such as sodium dodecyl sulfate and Triton X-100 abolished corncob formation. On the basis of these results, no additional salts or reagents were added to the standard assay buffer.

Effect of heat and protease treatments on corncob formation. The *F. nucleatum* strains were heated at 70°C for various periods of time and then assayed for corncob formation with untreated *S. sanguis* CC5A (Fig. 5). After being heated for 30 min, strain 10953 showed 86% inhibition of corncob formation, whereas strain 364 reached a maximum value of inhibition (54%) after only 5 min of heating. Heat treatment of *S. sanguis* CC5A resulted in a maximum inhibition of corncob formation 5 min after exposure; a 32% inhibition was seen with strain 364 and 35% with strain 10953. *F. nucleatum* 364 and 10953 were also treated with trypsin, followed by trypsin inhibitor, and assayed for corncob activity (Fig. 6A). Rapid inhibition of corncob formation was obtained after trypsin treatment for 30 min, and corncob formation was inhibited between 50 and 60% for strains 364 and 10953. Trypsin also abolished the corncob activity of *S.*

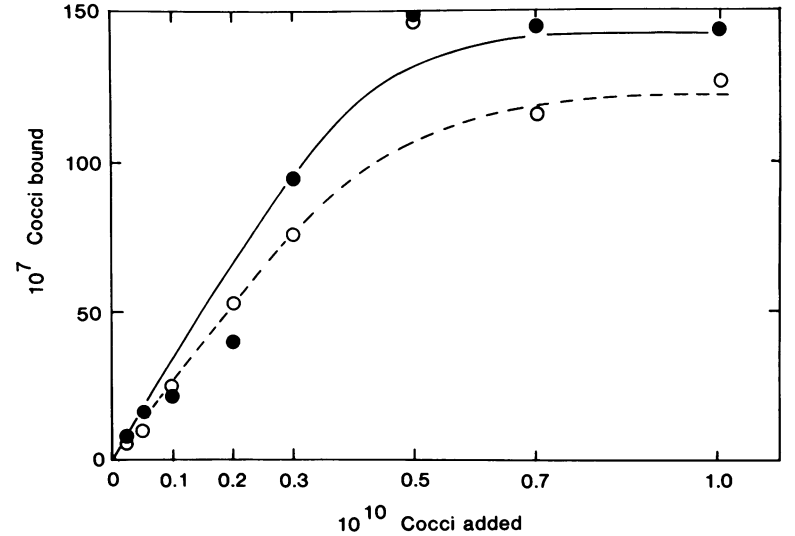

FIG. 4. Number of *S. sanguis* CC5A bound to a constant amount of *F. nucleatum* as a function of the number of cocci added. Symbols are explained in the legend to Fig. 3.

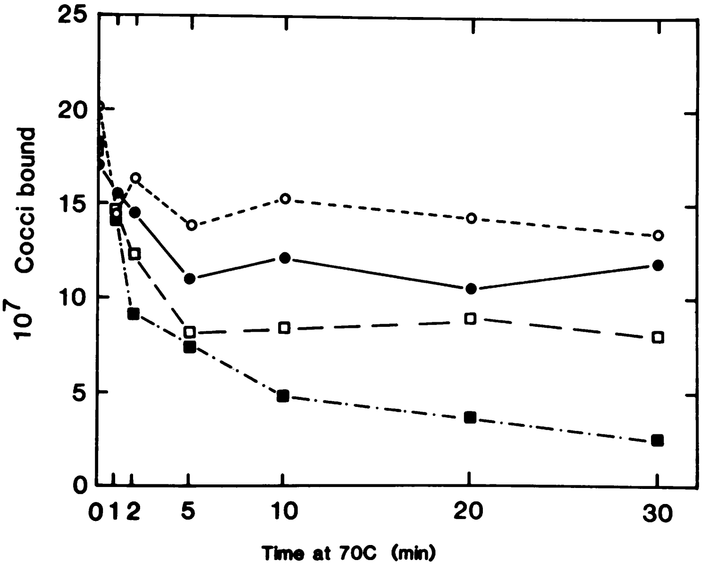

FIG. 5. Effects of heat treatment on corncob formation. Open squares, heat-treated *F. nucleatum* 364 assayed with untreated *S. sanguis* CC5A; closed squares, heat-treated *F. nucleatum* 10953 assayed with untreated CC5A; open circles, treated *S. sanguis* CC5A assayed with untreated *F. nucleatum* 364; closed circles, treated *S. sanguis* CC5A assayed with untreated *F. nucleatum* 10953.

sanguis CC5A just as rapidly; 50% inhibition was observed with untreated strain 364, and 76% inhibition was observed with untreated strain 10953 (Fig. 6B).

Although the results of the heat and trypsin experiments suggested that protein(s) or molecules associated with surface protein might be involved in corncob formation, they also suggested different sensitivities between the strains of *F. nucleatum* tested.

Relationship of corncob formation to in vitro plaque development. Corncobs were also formed

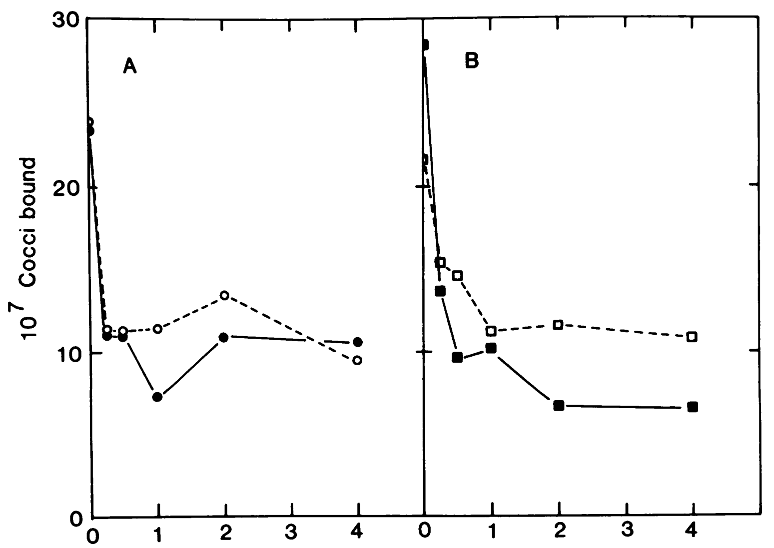

FIG. 6. Effects of trypsin treatment on corncob formation. Cells were treated with 0.5 mg of trypsin per ml, followed by 0.5 mg of trypsin inhibitor per ml. (A) Trypsin-treated *F. nucleatum* assayed with untreated *S. sanguis* CC5A. (B) Trypsin-treated CC5A assayed with untreated *F. nucleatum*. Open circles and squares, *F. nucleatum* 364; closed circles and squares, *F. nucleatum* 10953.

with *S. sanguis* G9B, which has been used extensively in studies of in vitro plaque formation (1). To determine the effect of corncob formation on plaque formation, the *F. nucleatum* strains were mixed with ^{3}H-labeled *S. sanguis* G9B in the presence of saliva-coated hydroxyapatite, and adhesion was compared with that of controls in the absence of the fusobacteria. The results indicated a 60% increase in *S. sanguis* G9B adhesion in the presence of *F. nucleatum* 364 and a 178% increase in adhesion when strain G9B was mixed with strain 10953.

DISCUSSION

There are some similarities and differences between corncob formation involving *Bacterionema* sp. and *Fusobacterium* spp. The similarities are in their morphological resemblance to each other, their relative tolerance to ionic strength and various cations, and their sensitivity to EDTA. The *Bacterionema* model exhibited a distinct optimum at pH 6.5, whereas corncob formation in the *Fusobacterium* system increased as the pH was increased to 8, above which streptococcal aggregation interfered with the measurement of corncob formation. The *Bacterionema* corncobs seem to be relatively insensitive to detergents, whereas *Fusobacterium* corncobs are markedly inhibited by sodium dodecyl sulfate and Triton X-100.

Although morphologically both systems appear to be similar in that attachment of the streptococci to the filaments appears to be mediated through the localized fimbriae on *S. sanguis* CC5A, strains such as *S. sanguis* G9B, which do not appear to have such localized tufts, can form corncobs with fusobacteria but not with *Bacterionema* sp. However, the failure to detect localized tufts of fimbriae in some strains may be due to technical difficulties. Attempts to overcome these problems are being investigated, since it has been reported that such localized tufts are much more common than formerly thought (P. Handley, personal communication). Indeed, Handley has suggested that at least four morphologically distinct types of localized tufts are found in *S. sanguis*. However, differences in the conditions between corncob formation in *Bacterionema* sp. and *Fusobacterium* spp. suggest that different surface polymers or binding sites are involved.

In contrast to the *Bacterionema* model, trypsin and heat treatment of fusobacteria result in a reduction of corncob formation; similar treatments of *Bacterionema* sp. do not inhibit aggregate formation. Although trypsin treatment of the streptococci does reduce corncob formation in both systems, inhibition in the *Bacterionema* model is greater. Mouton et al. (16) have shown that lipoteichoic acid is present in the localized tufts of *S. sanguis* CC5A, and their studies, as well as those of others, have suggested that such fimbriae may also contain protein and carbohydrate (17). If the lipoteichoic acids are intimately associated with fimbriae proteins, as suggested by Ofek et al. (18), then trypsin treatment might not only remove surface proteins but could also solubilize the associated teichoic acids. Thus, these experiments still leave open the possibility that both protein and teichoic acids may play a role in corncob formation. Whatever the nature of the surface structures which are ultimately found to be involved in the process, our studies suggest that corncob formation is a more general phenomenon and may indeed be one of the mechanisms allowing for a change of the characteristic gram-positive supragingival plaque to a more gram-negative filamentous subgingival plaque.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grants IF-32-DE-05215 (P.L.), DE-02623 (J.M.D.), DE-03180 (B.R.), and DE-05123 (S.C.H.) from the National Institute of Dental Research.

LITERATURE CITED

1. **Applebaum, B., E. Golub, S. C. Holt, and B. Rosan.** 1979. In vitro studies of dental plaque formation: adsorption of oral streptococci to hydroxyapatite. Infect. Immun. **25:**717–728.
2. **Bourgeau, G., and B. C. McBride.** 1976. Dextran-mediated interbacterial aggregation between dextran-synthesizing streptococci and *Actinomyces viscosus*. Infect. Immun. **13:**1228–1234.
3. **Carlsson, J.** 1967. Presence of various types of nonhemolytic streptococci in dental plaque and in other sites in the oral cavity in man. Odontol. Revy **18:**55–74.
4. **Carlsson, J., H. Grahnen, and G. Jonsson.** 1975. Lactobacilli and streptococci in the mouths of children. Caries Res. **9:**333–339.
5. **Carlsson, J., H. Grahnen, G. Jonsson, and S. Wikner.** 1970. Establishment of *Streptococcus sanguis* in the mouths of infants. Arch. Oral Biol. **15:**1143–1148.
6. **Ellen, R. P., and I. P. Balcerzak-Raczkowski.** 1977. Interbacterial aggregation of *Actinomyces naeslundii* and dental plaque streptococci. J. Periodontal Res. 12:11–20.
7. **Gibbons, R. J., and N. Nygaard.** 1970. Interbacterial aggregation of plaque bacteria. Arch. Oral Biol. **15:**1397–1400.
8. **Jones, S. J.** 1972. A special relationship between spherical and filamentous micro-organisms in mature human dental plaque. Arch. Oral Biol. **17:**613–616.
9. **Lancy, P., Jr., B. Appelbaum, S. C. Holt, and B. Rosan.** 1980. Quantitative in vitro assay for "corncob" formation. Infect. Immun. **29:**663–670.
10. **Listgarten, M. A., H. Mayo, and M. Amsterdam.** 1973. Ultrastructure of the attachment device between coccal and filamentous microorganisms in "corncob" formations of dental plaque. Arch. Oral Biol. **18:**651–656.
11. **Listgarten, M. A., H. E. Mayo, and R. Tremblay.** 1975. Development of dental plaque on epoxy resin crowns in man. J. Periodontol. **46:**10–25.
12. **McIntire, F. C., A. E. Vatter, J. Baros, and J. Arnold.** 1978. Mechanism of coaggregation between *Actinomyces viscosus* T14V and *Streptococcus sanguis* 34. Infect. Immun. **21:**978–988.
13. **Moore, W. E. C., R. R. Ranney, and L. V. Holdeman.** 1982. Subgingival microflora in periodontal disease: cul-

tural studies, p. 13–26. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.

14. **Mouton, C.** 1974. Association bacterienne de types differents au sein de la plaque dentaire: la formation en epi de mais. J. Biol. Buccale **2**:207–224.
15. **Mouton, C., H. S. Reynolds, and R. J. Genco.** 1977. Combined micromanipulation, culture and immunofluorescent techniques for isolation of the coccal organisms comprising the "corn-cob" configuration of human dental plaque. J. Biol. Buccale **5**:321–332.
16. **Mouton, C., H. S. Reynolds, and R. J. Genco.** 1980. Characterization of tufted streptococci isolated from the "corncob" configuration of human dental plaque. Infect. Immun. **27**:235–245.
17. **Newman, H. N., and G. S. McKay.** 1973. An unusual microbial configuration in human dental plaque. Microbios **8**:117–128.
18. **Ofek, I., W. A. Simpson, and E. H. Beachey.** 1982. Formation of molecular complexes between a structurally defined M protein and acylated or deacylated lipoteichoic acid of *Streptococcus pyogenes*. J. Bacteriol. **149**:426–433.
19. **Poirier, T. P., S. J. Tonell, and S. C. Holt.** 1979. Ultrastructure of gliding bacteria: scanning electron microscopy of *Capnocytophaga sputigena*, *Capnocytophaga gingivalis*, and *Capnocytophaga ochracea*. Infect. Immun. **26**:1146–1158.
20. **Reynolds, E. J.** 1963. The use of lead citrate at high pH as an electron opaque stain for electron microscopy. J. Cell Biol. **17**:208–212.
21. **Ritz, H. L.** 1967. Microbial population shifts in developing human dental plaque. Arch. Oral Biol. **12**:1561–1568.
22. **Rosan, B., B. Appelbaum, L. K. Campbell, K. W. Knox, and A. J. Wicken.** 1982. Chemostat studies of the effect of environmental control on *Streptococcus sanguis* adherence to hydroxyapatite. Infect. Immun. **35**:64–70.
23. **Rosan, B., C. H. Lai, and M. A. Listgarten.** 1976. *Streptococcus sanguis*: a model in the application in immunological analysis for the *in-situ* localization of bacteria in dental plaque. J. Dent. Res. **55A**:124–141.
24. **Schachtele, C. F., S. K. Harlander, D. W. Fuller, P. K. Zollinger, and W. L. S. Leung.** 1976. Bacterial interference with sucrose dependent adhesion of oral streptococci, p. 401–412. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts), vol. II. Information Retrieval, Inc., Washington, D.C.
25. **Slots, J., and R. J. Gibbons.** 1978. Attachment of *Bacteroides melaniogenicus* subsp. *asaccharolyticus* to oral surfaces and its possible role in colonization of the mouth and of periodontal pockets. Infect. Immun. **19**:254–264.
26. **Takazoe, I., T. Matsukubo, and T. Kato.** 1978. Experimental formation of "corn-cob" *in vitro*. J. Dent. Res. **57**:384–387.
27. **van Houte, J.** 1976. Oral bacterial colonization: mechanisms and implications, p. 3–32. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts), vol. I. Information Retrieval, Inc., Washington, D.C.